Short communication

Introducing a novel bacterium, *Vibrio neocaledonicus* sp., with the highest corrosion inhibition efficiency

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ABSTRACT

Degradation of metals due to corrosion causes serious economic problems throughout the world, and different corrosion protection techniques are being used to extend the service life of metallic structures. It has been suggested that some microorganisms can inhibit electrochemical corrosion of metals. Here we isolated a new marine inhibitory bacterium, *Vibrio neocaledonicus* sp., and EIS results showed that the corrosion resistance of carbon steel increased by more than sixty fold in the presence of this bacterium. This is the highest corrosion inhibitory effect reported for bacteria and is comparable with some industrial coatings such as electroless Ni. This bacterium affected corrosion by the formation of an inhibitory layer on the metal surface in the first hours of attachment, with the consumption of oxygen by electron transport proteins. Extracellular polymeric substances produced by this bacterium also have a corrosion inhibitory effect. Thus we propose a new, natural, non-toxic, and cost-effective system for controlling corrosion processes using this bacterium or extracellular polymeric substances produced by this bacterium.

1. Introduction

Marine steel structures, such as harbor and oil exploration facilities, become corroded over time and need to be renovated or modified, which is expensive. Currently, bio-based anti-corrosion agents are new substitutes for conventional products which are composed of biological products [1–3].

Due to the high interest for the application of natural, non-toxic and effective environmentally friendly materials as corrosion inhibitors instead of biocides or toxic evaporative organic compounds, the use of bacteria and their metabolic by-products including biofilm and extracellular polymeric substances (EPSs) is considered a lot. It has been suggested that bacteria and their metabolic by-products can eliminate corrosion-causing parameters, possibly due to the formation of biofilms that use up the oxygen which would otherwise be available to oxidize that metal [4–7].

The first report on the corrosion inhibitory effect of bacteria was by Pedersen et al. [8]. Later, Jayaraman et al. [9] studied the mechanism underlying this process and reported that biofilm formation was crucial. A variety of bacteria have been isolated and their corrosion inhibitory effects were examined [10–13]. The highest inhibitory effect of tenfold was found for *Pseudomonas mendocina* KR1 [6]. Different mechanisms have been proposed to explain corrosion inhibition by bacteria such as oxygen depletion on the metal surface because of biofilm formation [14]; formation of inorganic materials [15]; generation of antimicrobials by biofilms [16]; production of biofilm-secreted biosurfactant [17]; and corrosion inhibition by bacteriophages [18].

In this study, we present a new marine inhibitory bacterium, *Vibrio neocaledonicus* sp., with the highest corrosion inhibitory effect reported for bacteria. The mechanism and efficiency of corrosion inhibition were studied by different electrochemical, surface analysis and spectroscopic methods. To confirm the inhibitory effect of bacterium metabolic byproducts, EPS produced by this bacterium was extracted and examined.

2. Materials and methods

2.1. Isolation and identification

Marine *V. neocaledonicus* sp. KJ841877 was isolated from marine sludge that was collected from the East Sea. Sampling, strain isolation, and culture procedures have been described previously [19]. This bacterium is deposited as MCCC 1K00266 in the Marine Culture Collection of

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China (MCCC), Xiamen, China. A strain culture is stored at −80 °C in marine broth (MB) that contained 30% glycerol.

2.2. Materials

Squared-shaped carbon steel (ASTM A36) specimens with sides 15 mm thickness of 1 mm were used. A36 carbon steel (CS) is composed of 0.20 wt.% C, 1.4 wt.% Mn, 0.35 wt.% Si, 0.045 wt.% S, and 0.045 wt.% P with the remainder Fe. The method for creating working electrodes has been described elsewhere [20]. The surfaces were sterilized with UV light and pure ethanol for 1 h before experiments.

Artificial seawater [21] was used throughout the corrosion studies, and approximately 3 g/L of peptone was added to this medium as a carbon and energy source. Then, the solution was autoclaved at 120 °C for 20 min. Before performing electrochemical experiments, the metal samples were placed in artificial seawater under sterile conditions and in the presence of bacteria in an aerobic chamber. The samples were then incubated at 30 °C on a rotary shaker at 80 rpm. During electrochemical measurements, samples were immersed in a water bath to maintain the temperature at 30 °C.

2.3. Electrochemical studies

Electrochemical tests were performed in a corrosion cell with a three-electrode system using an AUTOLAB Potentiostat/Galvanostat (PGSTAT302). Electrochemical impedance spectroscopy (EIS) was performed at a steady state open circuit potential (E_{OCP}) with an AC sine wave of amplitude 10 mV at frequencies ranging from 100 kHz to 10 MHz. Potentiodynamic polarization curves were then obtained at a scan rate of 0.l66 mV/s. GPES was used to acquire data and analyze potentiodynamic polarization curve data; FRA with ZSIMPWIN software was used for EIS data analysis. Experiments were performed three times for each condition used, and standard deviations (SDs) were also calculated to determine the precision and repeatability of electrochemical results.

2.4. Surface observation and characterization

The surface characteristics and corrosion features of CS specimens were examined by field emission scanning electron microscopy (FESEM) after the specimens were exposed to artificial seawater without and with bacteria for different times. Before experiments, bacterial cells were fixed and dehydrated by the immersion of the samples in 2% glutaraldehyde solution for 4 h and dehydration by four ethanol solutions (15 min each): 25%, 50%, 75% and 100%. Fourier transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS) analyses were used for surface characterization. The test metal samples were exposed to sterile artificial seawater either without or with bacteria for 10 days. Then, the samples were dried and examined structurally using FTIR and XPS. To analyze the bacterium-produced EPS, pellets for infrared analysis were obtained by grinding a mixture of 2 mg of polysaccharide with 200 mg of dry KBr, followed by pressing into a 16-mm diameter mold. FTIR spectra were recorded with a resolution of 4 cm^{-1} in the 4000–400 cm^{-1} region. XPS measurements were estimated using an AXIS ULTRA DLD spectrometer (Shimadzu, Japan) with a monochromatized Al K_α X-ray source ($h\nu = 1486.6$ eV) and an X-ray beam of around 1 mm. Survey spectra were recorded for samples in the 0–1100 eV kinetic energy range using 1 eV steps and high resolution scans were performed using 0.1 eV steps over the following regions of interest: C 1s, N 1s, O 1s and Fe 2p. Quantification of outer layer atomic compositions and spectral simulation of the experimental peaks were achieved using software provided by VG Scientific. Dried EPS was compressed into a pellet and attached to a carbon tape for XPS analysis.

2.5. EPS production

For EPS production, the bacteria were grown in artificial seawater that included 5 g/L of peptone, 1 g/L of yeast extract and 30 g/L of glucose. The pH of the medium was adjusted to 7.5 with saturated NaOH. The medium was sterilized by autoclaving at 120 °C for 20 min and was inoculated with 2 vol.% 18-h-old culture grown in the same medium at room temperature on a rotary shaker at 150 rpm. Then, the culture was centrifuged at 15,000 ×*g* at 4 °C for 20 min. The supernatants were pressure filtered through cellulose nitrate filters (pore size 0.25 μm). The supernatant liquid was then transferred to a new Oak Ridge tube. Adding isopropanol effectively lowered the solubility of polymers and caused them to precipitate. Centrifugation (7000 ×*g* for 5 min) was used to separate the liquid supernatant from the precipitate, which was dried at 30 °C to remove any residual isopropanol [22].

3. Results and discussion

3.1. V. neocaledonicus sp. and its corrosion inhibition efficiency

A new generation of *Vibrio* strain, *V. neocaledonicus* sp. KJ841877 was isolated from the East Sea (Ningbo, China, GPS 29° 59′ 50.59″ N, 122° 2′ 34.99″ E) and after submitted to GenBank, deposited as MCCC 1K00266 in Marine Culture Collection of China (MCCC), Xiamen, China. This bacterium is a genus of Gram-negative bacteria that are typically found in saltwater. These bacteria have developed various strategies to survive under extreme conditions, such as metabolic pathway adaptations including the production of protective structures like biofilms [23].

The corrosion inhibitory effect was investigated using electrochemical techniques. The results showed that the impedance of specimens exposed only to sterile artificial seawater was approximately 830 $\Omega \cdot cm^2$ and increased to 1311 $\Omega \cdot cm^2$ after one month. However, in the presence of the bacteria, the impedance value was nearly 40,700 $\Omega \cdot cm^2$ after 24 h and increased to 49,000 $\Omega \cdot cm^2$ after one month. Thus, this type of bacterium increased impedance up to sixty fold after one month (Fig. 1a), and thereafter, the impedance remained relatively stable. This capacity is comparable with some industrial coatings such as electroless Ni–P or Ni–P–PTFE [24,25].

Potentiodynamic polarization measurements showed that the corrosion current density (i_{corr}) decreased significantly for samples exposed to the bacteria. As can be seen in Fig. 1b, the cathodic curves shifted toward a lower current density in the presence of bacteria, confirming a decrease in O_2 reduction reaction, as bacteria generally use O_2 to oxidize nutrients and obtain energy. In addition, the bacterium retarded the rate of the anodic process, probably through the mechanism of adsorption [26]. The electrochemical values were obtained by the extrapolation of the linear Tafel segments. The results showed that the corrosion current density of CS decreased from 29.59 μA/cm^2 to 1.24 μA/cm^2 after 24 h and reached to 0.94 μA/cm^2 after 10 days of exposure to the sterile artificial seawater containing bacteria. However, the corrosion current density value of CS exposed to the sterile artificial for 10 days was 8.56 μA/cm^2. In addition, a significant negative shift in corrosion potential from −732 mV (vs SCE) to −938 mV (vs SCE) was observed in sterile artificial seawater after 10 days due to the continuous degradation and dissolution of the metal [27] and the attack of the chloride ions on the CS surface; while in the presence of bacterium, corrosion potential is shifted toward less negative potentials (60 mV) and reached to −792 mV (vs CSE) after 10 days.

To verify the effect of *V. neocaledonicus* sp. KJ841877 on real systems, we prepared some CS coupons and exposed them to seawater without and with bacteria for one month. The photographic images illustrate a thick rust layer on the surface exposed only to seawater (Fig. 3e), whereas a clean-looking inhibitory film formed due to the bacteria was attached to the surface after one month in the presence of bacteria (Fig. 3f).

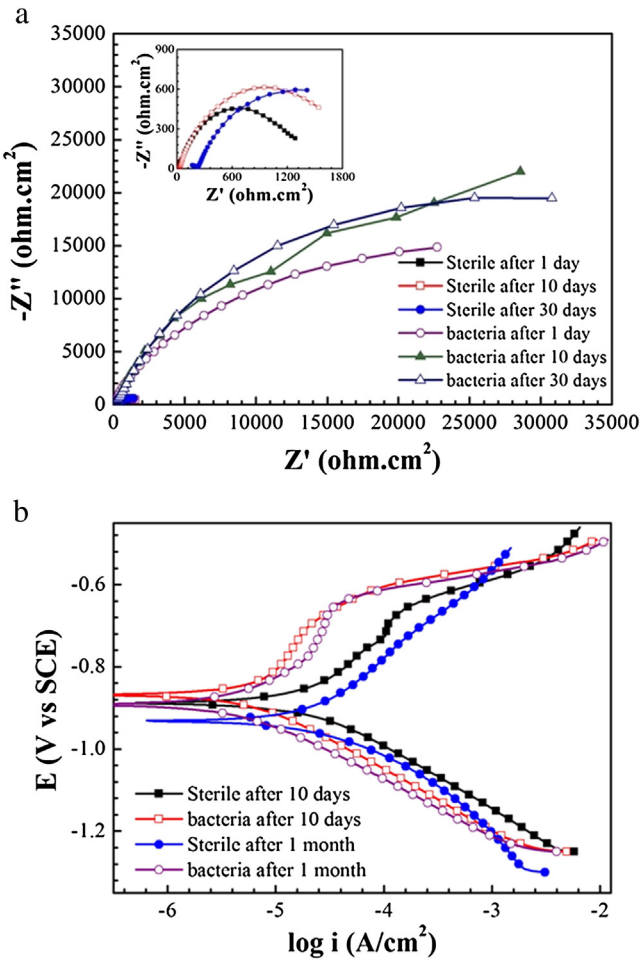

Fig. 1. (a) Comparisons of impedance values for CS specimens in sterile artificial seawater without and with bacteria after exposure for 24 h, 10 days and 1 month. Impedance value increased forty fold after 24 h and reached up to sixty fold of that with sterile artificial seawater after 1 month. Moreover, impedance remained stable over time if the growth medium was refreshed. (b) Comparisons of potentiodynamic polarization curves for CS specimens after 10 days and 1 month exposed to sterile artificial seawater without and with bacteria in a shaker incubator (80 rpm) at 30 °C. The cathodic and anodic branches were nearly similar for both solutions.

3.2. Mechanism of bacterium and their metabolic byproducts on the corrosion inhibition of carbon steel

To study the mechanism of the corrosion inhibitory effect of bacterium, surface analysis methods such as FTIR, XPS and FESEM were used. FTIR spectrum of a CS sample surface exposed only to sterile artificial seawater (Fig. 2A) revealed two peaks at 3400 cm^{-1} and 484.8 cm^{-1}, which correspond to absorbed water molecules and γ-Fe_2O_3. In the presence of samples exposed to seawater with the bacteria, four main peaks were detected: 1638.9 cm^{-1}, 1493.6 cm^{-1}, 1130.4 cm^{-1} and 854.4 cm^{-1}, corresponding to iron phosphate, the C═O bond of amide I, polysaccharides and C – O stretching in esters [28], respectively. Schmitt and Flemming identified the spectral range of 1150–750 cm^{-1} as the polysaccharide region [16], so, it appears the polysaccharide was produced by these bacteria and formed complexes with the metal. Reducing the amount of electron acceptors at the interface by binding EPS with Fe(II) and Fe(III) inhibits corrosion [29].

FTIR spectra of EPS extracted from bacterium showed adsorption bands at 1641.8 cm^{-1} and 1401 cm^{-1}, corresponding to the asymmetric and symmetric stretching of carboxylates in the side chains [30]. A strong-intensity band caused by the stretching vibration of C – O – C in the backbone of polysaccharides was also found at 1112.7 cm^{-1} [31]. Based on these observations, the EPS excreted by this bacterium

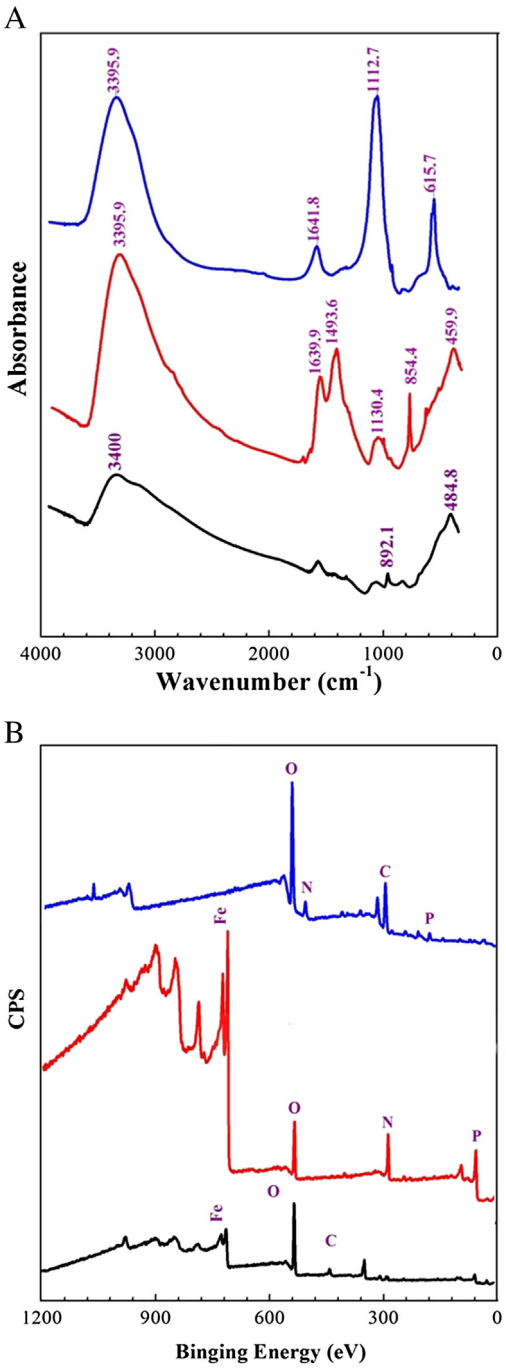

Fig. 2. (A) FTIR spectra of CS in sterile artificial seawater (black line), artificial seawater that contained bacteria (red line) after exposure for 10 days and EPS extracted from bacteria (blue line). The wave numbers of the major peaks are shown in these plots. The maximum intensity of the absorption band at approximately 1112.7 cm^{-1} (indicating stretching vibration of C – O – C) was obtained with EPS. (B) XPS survey spectra of CS exposed to sterile artificial seawater (black line), artificial seawater that contained bacteria (red line) for 10 days, and XPS spectra of EPS formed by bacteria (blue line). All spectra were measured at Θ = 45°. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

primarily consisted of exopolysaccharides with numerous hydroxyl and carboxyl groups. Amide groups from proteins were also observed in the FTIR peaks. Organic macromolecular compounds, such as proteins and carbohydrates, can spontaneously adsorb onto a solid surface and form an inhibitory layer after a short exposure time [31], so our results are consistent with free EPS released into bulk solution to compete with bacterial cells for binding sites on the metal surface [32].

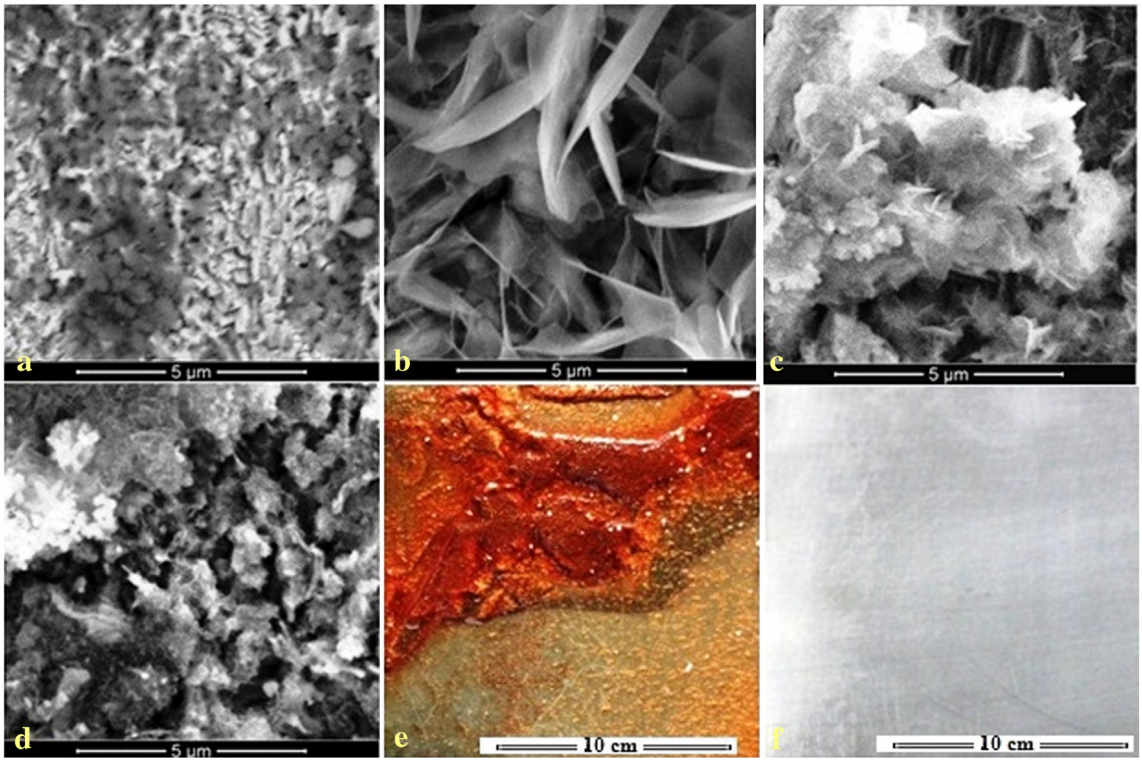

Fig. 3. FESEM images of CS exposed to sterile artificial seawater without and with bacteria for 24 h (a, c), and 10 days (b, d). EPS produced a conditioning layer on the metal surface after 6 h. This layer became thicker and formed a biofilm on the metal surface after 10 days. By comparison, when in a sterile solution, an oxide layer covered the entire surface. Photographs of CS coupons exposed to seawater without (e) and with bacteria (f) for one month show a thick rust layer on surface without bacteria.

The XPS spectrum (Fig. 2b) of the CS surface showed a higher percentage of C and Fe in the presence of the bacteria which indicate the formation of Fe–EPS complexes. In contrast, oxygen from the formation of oxide layers was found on CS surfaces exposed solely to sterile artificial seawater.

The microstructure of the CS surfaces and inhibitive layer were examined by FESEM. Metal surfaces exposed only to sterile artificial seawater were covered by an oxide layer with large craters after 24 h (Fig. 3a), this layer heterogeneously covered the surface depending on the exposure time (Fig. 3b). In the presence of bacteria, an intact film layer extensively coated the metal surface after 6 h (Fig. 3c) and became thicker with longer exposure time (Fig. 3d).

3.3. The effect of EPS produced with bacterium on the corrosion inhibition of carbon steel

Similarly, we exposed CS samples in artificial seawater without and with 5 g/L of bacterium-produced EPS to examine the corrosion inhibition of EPS. This concentration was selected based on preliminary experiments to determine the optimum inhibitory concentration. The addition of EPS increased the impedance value and inhibited corrosion. The inhibitory effect could be attributed to EPS adsorption at the steel/solution interface. The corrosion rate also decreased in correlation with a shift in the anodic branch of the polarization curves toward lower current densities. This suggests that EPS behaved as an inhibitive layer and blocked the active sites available for corrosion reaction [33], and the formation of inhibitory Fe–EPS complex layers on the metal surface was the main reason for the corrosion inhibition of the bacteria [6]. The cathodic reaction also decreased, due to oxygen consumption by the bacteria, and this would reduce the corrosion rate. The presence of carbohydrates and amide groups in the EPS and inhibitive layers suggests that EPS could adheres to metal surfaces by these functional groups and decreases the corrosion process by retarding the anodic reaction [34].

4. Conclusion

The study of the corrosion inhibitory effect of *V. neocaledonicus* sp. and EPS produced by this bacterium showed that the bacterium and its metabolic byproduct have a high inhibitory effect against corrosion of carbon steel. The inhibitory effect was caused by the formation of an inhibitory layer which covers the entire metal surfaces. The layer is composed of Fe–EPS complexes and strengthens by exposure time. We propose that *V. neocaledonicus* sp. and its EPS can be used as a new, natural, non-toxic, and cost-effective system for controlling corrosion processes of carbon steel.

Conflict of interest

There is no conflict of interest.

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References

[1] H.T. Dinh, J. Kuever, M. Mumann, A.W. Hassel, M. Stratmann, F. Widdel, Iron corrosion by novel anaerobic microorganisms, Nature 427 (2004) 829–832.
[2] M. Felix, J.E. Martin-Alfonso, A. Romero, A. Guerrero, Development of albumen/soy biobased plastic materials processed by injection molding, J. Food Eng. 125 (2014) 7–16.
[3] F. Tang, X. Wang, X. Xu, L. Li, Phytic acid doped nanoparticles for green anticorrosion coatings, Colloid. Surf. A. 369 (2010) 101–105.
[4] A. Pedersen, M. Hermansson, The effects on metal corrosion by *Serratia marcescens* and a *Pseudomonas* sp. Biofouling 1 (1989) 313–322.
[5] A. Pedersen, M. Hermansson, Inhibition of metal corrosion by bacteria, Biofouling 3 (1991) 1–11.

[6] A. Jayaraman, E.T. Cheng, J.C. Earthman, T.K. Wood, Axenic aerobic biofilms inhibit corrosion of SAE1018 steel through oxygen depletion, Appl. Microbiol. Biotechnol. 48 (1997) 11–17.
[7] A. Jayaraman, E.T. Cheng, J.C. Earthman, T.K. Wood, Importance of biofilm formation for corrosion inhibition of SAE 1018 steel by axenic aerobic biofilms, J. Ind. Microbiol. 18 (1997) 396–401.
[8] A. Pedersen, S. Kjelleberg, M.A. Hermansson, Screening method for bacterial corrosion of metals, J. Microbiol. Methods 8 (1988) 191–198.
[9] A. Jayaraman, J.C. Earthman, T.K. Wood, Corrosion inhibition by aerobic biofilms on SAE 1018 steel, Appl. Microbiol. Biotechnol. 7 (1997) 62–68.
[10] R. Zuo, Biofilms: strategies for metal corrosion inhibition employing microorganisms, Appl. Microbiol. Biotechnol. 76 (2007) 1245–1253.
[11] D. Ornek, A. Jayaraman, B.C. Syrett, C.H. Hsu, F.B. Mansfeld, T.K. Wood, Pitting corrosion inhibition of aluminum 2024 by *Bacillus* biofilms secreting polyaspartate or gamma-polyglutamate, Appl. Microbiol. Biotechnol. 58 (2002) 651–657.
[12] A. Jayaraman, F.B. Mansfeld, T.K. Wood, Inhibiting sulfate reducing bacteria in biofilms by expressing the antimicrobial peptides indolicidin and bactenecin, J. Ind. Microbiol. Biotechnol. 22 (1999) 167–175.
[13] A. Jayaraman, J.C. Earthman, T.K. Wood, Corrosion inhibition by aerobic biofilms on SAE 1018 steel, Appl. Microbiol. Biotechnol. 47 (1997) 62–68.
[14] H.P. Volkland, H. Harms, B. Muller, G. Repphun, O. Wanner, A.J.B. Zehnder, Bacterial phosphating of mild (unalloyed) steel, Appl. Environ. Microbiol. 66 (2000) 4389–4395.
[15] E. Juzeliunas, R. Ramanauskas, A. Lugauskas, M. Samuleviciene, K. Leinartas, Microbially influenced corrosion acceleration and inhibition. EIS study of Zn and Al subjected for two years to influence of *Penicillium frequentans*, *Aspergillus niger* and *Bacillus mycoides*, Electrochem. Commun. 7 (2005) 305–311.
[16] J. Schmitt, H.C. Flemming, FTIR-spectroscopy in microbial and material analysis, Int. Biodeterior. Biodegrad. 41 (1998) 1–11.
[17] C. Dagbert, T. Meylheuc, M.N. Bellon-Fontaine, Corrosion behaviour of AISI 304 stainless steel in presence of a biosurfactant produced by *Pseudomonas fluorescens*, Electrochim. Acta 51 (2006) 5221–5227.
[18] H.S.C. Eydal, S. Jagevall, M. Hermansson, K. Pedersen, Bacteriophage lytic to *Desulfovibrio aespoeensis* isolated from deep groundwater, ISME J. 3 (2009) 1139–1147.
[19] M. Moradi, J. Duan, H. Ashassi-Sorkhabi, X. Luan, De-alloying of 316 stainless steel in the presence of a mixture of metal-oxidizing bacteria, Corros. Sci. 53 (2011) 4282–4290.
[20] M. Moradi, Z. Song, L. Yang, J. Jiang, J. He, Effect of marine *Pseudoalteromonas* sp. on the microstructure and corrosion behaviour of 2205 duplex stainless steel, Corros. Sci. 84 (2014) 103–112.
[21] D.R. Kester, I.W. Duedall, D.N. Connors, R.M. Pytkowicz, Preparation of artificial seawater, Limnol. Oceanogr. 12 (1967) 176–179.
[22] P.V. Bramhachari, S.K. Dubey, Isolation and characterization of exopolysaccharide produced by *Vibrio harveyi* strain VB23, Lett. Appl. Microbiol. 43 (2006) 571–577.
[23] F.L. Thompson, D. Gevers, C.C. Thompson, P. Dawyndt, S. Naser, B. Hoste, C.B. Munn, J. Swings, Phylogeny and molecular identification of *Vibrios* on the basis of multilocus sequence analysis, Appl. Environ. Microbiol. 71 (2005) 5107–5115.
[24] H.L. Wang, L.Y. Liu, Y. Dou, W.Z. Zhang, W.F. Jiang, Preparation and corrosion resistance of electroless Ni–P/SiC functionally gradient coatings on AZ91D magnesium alloy, Appl. Surf. Sci. 286 (2013) 319–327.
[25] J. Wang, J. Tian, X. Liu, Y. Yin, X. Wang, Effect of polytetrafluoroethylene content on electrochemical anticorrosion behaviors of electroless deposited Ni–P and Ni–P–polytetrafluoroethylene coatings in seawater, Thin Solid Films 519 (2011) 5905–5911.
[26] C.M. Goulart, A. Esteves-Souza, C.A. Martinez-Huitle, C.J.F. Rodrigues, M.A.M. Maciel, A. Echevarria, Experimental and theoretical evaluation of semicarbazones and thiosemicarbazones as organic corrosion inhibitors, Corros. Sci. 67 (2013) 281–291.
[27] H. Amar, T. Braisaz, D. Villemin, B. Moreau, Thiomorpholin-4-ylmethyl-phosphonic acid and morpholin-4-methyl-phosphonic acid as corrosion inhibitors for carbon steel in natural seawater, Mater. Chem. Phys. 110 (2008) 1–6.
[28] M.D. Ghafari, A. Bahrami, I. Rasooli, D. Arabian, F. Ghafari, Bacterial exopolymeric inhibition of carbon steel corrosion, Int. Biodeterior. Biodegrad. 80 (2013) 29–33.
[29] V.L. Finkenstadt, G.L. Cote, J.L. Willett, Corrosion protection of low-carbon steel using exopolysaccharide coatings from *Leuconostoc mesenteroides*, Biotechnol. Lett. 33 (2011) 1093–1100.
[30] Q. Bao, D. Zhang, D. Lv, P. Wang, Effects of two main metabolites of sulphate-reducing bacteria on the corrosion of Q235 steels in 3.5 wt.% NaCl media, Corros. Sci. 65 (2012) 405–413.
[31] C.M. Pradier, P. Bertrand, M.N. Bellon-Fontaine, C. Compere, D. Costa, P. Marcus, C. Poleunis, B. Rondot, M.G. Walls, Adsorption of proteins on an AISI 316 stainless-steel surface in natural seawater, Surf. Interface Anal. 30 (2000) 45–49.
[32] H.J.A. Breur, J.H.W. de Wit, J. van Turnhout, G.M. Ferrari, Electrochemical impedance study on the formation of biological iron phosphate layers, Electrochim. Acta 47 (2002) 2289–2295.
[33] P.J. Antony, R.K.S. Raman, P. Kumar, Corrosion of 2205 duplex stainless steel weldment in chloride medium containing sulfate-reducing bacteria, Metall. Mater. Trans. A 39 (2008) 2689–2697.
[34] G. Gunasekaran, S. Chongdar, S.N. Gaonkar, P. Kumar, Influence of bacteria on film formation inhibiting corrosion, Corros. Sci. 46 (2004) 1953–1967.